UNIVERSIDADE FEDERAL DO PARANÁ
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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

CONTRIBUIÇÕES PARA A HISTOPATOLOGIA, FISIOLOGIA E CLÍNICA NA OFTALMOLOGIA DE AVES

CURITIBA
2015

THIAGO ALEGRE COELHO FERREIRA

CONTRIBUIÇÕES PARA A HISTOPATOLOGIA, FISIOLOGIA E CLÍNICA NA OFTALMOLOGIA DE AVES

Dissertação apresentada ao Programa de Pós-Graduação da Universidade Federal do Paraná para a obtenção do título de Mestre.
Orientador: Prof. Dr. Fabiano Montiani-Ferreira

CURITIBA
2015

PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

UFPR

Ata da Defesa de Dissertação do Candidato ao Título de Mestre em Ciências Veterinárias, **THIAGO ALEGRE COELHO FERREIRA**, área Ciências Veterinárias, do PPGCV realizada em 29.01.2015

Às quatorze horas do dia vinte e nove de janeiro do ano dois mil e quinze, no Anfiteatro do Hospital Veterinário do Setor de Ciências Agrárias da UFPR, reuniu-se a Comissão Examinadora constituída pelos seguintes membros Professor Dr. Fabiano Montiani Ferreira; Professor Dr. Rogério Ribas Lange e o professor Dr. José Ademar Villanova Junior com a finalidade de argüir o mestrando **THIAGO ALEGRE COELHO FERREIRA** candidato ao Título de Mestre em Ciências Veterinárias, área Ciências Veterinárias, que ofereceu para análise da Comissão a Dissertação intitulada **"CONTRIBUIÇÕES PARA A HISTOPATOLOGIA, FISIOLOGIA E CLÍNICA NA OFTALMOLOGIA DE AVES".** Abertos os trabalhos o candidato, cumprindo determinação regimental, fez uma breve exposição oral a respeito de sua Dissertação. Terminada a exposição, o Presidente Professor Doutor Fabiano Montiani Ferreira declarou aberta a argüição do candidato pelos membros da banca, finalizada pelo próprio Presidente. Concluída a argüição, a Comissão Examinadora reuniu-se para avaliar o Candidato. A Comissão Examinadora considerou que a Dissertação ATENDE AOS REQUISITOS DE UM PROGRAMA DE MESTRADO.

Quanto à apresentação do Candidato durante a Defesa, a Comissão Examinadora CONSIDEROU QUE O CANDIDATO SATISFEZ TODAS AS QUESTÕES PROPOSTAS.

Reabertos os trabalhos, de acordo com o Art. 79 da Resolução nº 65/09–CEPE, o candidato foi considerado **APTO** para obtenção do título de Mestre em Ciências Veterinárias, Área Ciências Veterinárias, encerrando os trabalhos da Defesa de Dissertação dos quais, eu, FABIANO MONTIANI, lavrei a presente Ata que vai por mim assinada _____ e por todos os Membros da Comissão Examinadora. Curitiba, 29 de janeiro de 2015.

Professor Dr. Fabiano Montiani Ferreira
Presidente/Orientador

Professor Dr. Rogério Ribas Lange
Membro

Professor Dr. José Ademar Villanova Junior
Membro

“Tomei a decisão de fingir que todas as coisas que até então haviam entrado na minha mente não eram mais verdadeiras do que as ilusões dos meus sonhos”.

René Descartes

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RESUMO

No primeiro capítulo relata-se um caso clínico de um tumor retrobulbar de morfologia atípica em periquito-australiano (*Melopsittacus undulatus*). Na descrição desse caso foi empregada histopatologia e a técnica de imuno-histoquímica com quatro marcadores (anticorpos) em uma parceria entre o Laboratório de Oftalmologia Comparada (LABOCO) da UFPR e o Laboratório de Patologia Ocular Comparada de Wisconsin (*Comparative Ocular Pathology Laboratory of Wisconsin*- COPLOW). São raras as descrições detalhadas de tumores oculares em aves e praticamente inexistem relatos de tumores oculares nesta espécie em particular. Tais fatos fazem com que o prognóstico e a biologia dos tumores oculares nas aves ainda sejam praticamente desconhecidos. A investigação imuno-histoquímica obteve alguns resultados (marcação moderada por vimentina e negativa para as demais) que foram motivo de grande discussão tanto nesse relato, quanto para futuras pesquisas.

O segundo capítulo teve como objetivo estudar uma das incógnitas ainda por serem investigadas nessa área: a(s) função(ões) do *pecten*. O *pecten* é uma estrutura intraocular pigmentada que se projeta no corpo vítreo da região vizinha ao nervo óptico em direção à lente, constituída principalmente por um fino parênquima pigmentado e vasos sanguíneos que possuem trajetória sinuosa. Diferentes funções já foram atribuídas para esta estrutura, sabem-se também muitos detalhes da sua forma. Diversos trabalhos foram publicados que inclusive abrangem aspectos morfológicos do órgão por meio de microscopia óptica e eletrônica. Todavia, as pesquisas utilizando metodologias que visam explorar e focar na sua função, principalmente *in vivo*, ainda são escassas e muitas vezes inconclusivas. São particularmente raros os trabalhos sobre os vasos que originam a microvasculatura que compõe o *pecten*, sendo que o presente estudo é o pioneiro na avaliação de sua hemodinâmica. Nesse trabalho foi empregado um método não comumente utilizado na rotina clínica oftalmológica médica e veterinária, em marrecos-de-pequim (*Anas platyrhynchos domestica*), que é a ultrassonografia em modo Doppler. Trata-se de um exame não invasivo e indolor. A expectativa foi que tal exame fosse capaz de fornecer dados inéditos em relação à função do *pecten*.

Palavras-chave: oftalmologia, aves, neoplasias oculares, *pecten*, hemodinâmica, artéria *pectinis oculis*, Doppler colorido.

ABSTRACT

The first chapter reports the presence of a retrobulbar tumor morphologically intriguing in a budgerigar (*Melopsittacus undulatus*).In this case report histopathology and imunohistochemistry with four antibodies were used in a collaborative effort between the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW) and Comparative Ophthalmology Laboratory of UFPR (LABOCO). Detailed reports of ocular tumors in avian species are rare and practically do not exist in this particular specie. The absence of published reports creates a knowledge gap mainly in terms of prognosis and biology of ocular tumors in avian species. Immunohistochemistry technique obtained results (moderate positivity to vimentine and negative for others) that generated discussion both this case and future investigations.

The second chapter had the objective to study one of the remaining mysteries yet to be investigated in this area: the function of the *pecten oculi*. The *pecten*is a pigmented structure that projects in the vitreous body from the area adjacent to the optic nerve toward the lens, composed by a fine pigmented parenchyma and rich in blood vessels that possess a sinuous trajectory and only avian species possess. Different functions were attributed to this tissue and its structural composition was already described in detail. Several morphologic features of this structure were already published using both light and electron microscopy. Investigations using methods to explore the function of the pecten are scarce, mainly *in vivo*, while others are inconclusive. Investigations focusing on blood vessels that originate in the *pecten oculi* microvasculature are lacking. The present work is a pioneer investigating the hemodynamic aspects of the blood vessels present in the *pecten*. In this study color Doppler ultrasonography was used in pekin ducks (*Anas platyrhynchos domestica*). It is a noninvasive and painless diagnostic method not routinely used in the eye examination in veterinary and medical practice. The prospect was that results of this exam would be able to provide unpublished data and generate assumptions in relation to the function of the avian *pecten*.

Key words: ophthalmology, avian, ocular neoplasia, *pecten*, hemodynamics, *pectinisoculis* artery, color Doppler.

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LISTA DE ABREVIAÇÕES

ARVO – Association for Research in Vision and Ophthalmology

EDV – End Diastolic Velocity

IOP – Intraocular Pressure

LPCA – Long Posterior Ciliary Artery

PI – Pulsatility Index

PSV – Peak Systolic Velocity

RI – Resistive Index

TAMX - Time Average Maximal Velocity

TID – *Ter in die* (três vezes ao dia).

UFPR – Universidade Federal do Paraná

SUMÁRIO

1. OBJETIVOS

1.1 OBJETIVOS GERAIS

O objetivo da presente dissertação foi abordar assuntos ainda pouco investigados e pouco discutidos dentro da oftalmologia veterinária, com foco na oftalmologia aves. Realizamos estudos em duas espécies diferentes no âmbito de padronizar alguns parâmetros oftálmicos, e de pesquisar por meio de exames já comuns para outros fins, mas não os descritos nesse estudo, estruturas anatômicas nos olhos das espécies que serão descritas.

1.2 OBJETIVOS ESPECÍFICOS

- Relatar um caso de tumor mesenquimal maligno em um periquito-australiano (*Melopsittacus undulatus*).
- Expor a importância do envio de materiais biológicos oculares provenientes dos periquitos-australianos (*Melopsittacus undulatus*), para melhor entendimento das neoplasias que acometem os olhos e anexos nessa espécie.
- Determinar por meio de ultrassonografia, os parâmetros hemodinâmicos presentes na artéria pecteniana de marrecos-de-pequim (*Anas platyrhynchos domestica*).
- Comparar os valores encontrados com outros valores já estabelecidos para outros tecidos.
- Sugerir uma possível função para o pécten ocular de aves com base nos achados deste trabalho, analisando dados anatômicos já obtidos.

CAPÍTULO 1
TUMOR MESENQUIMAL MALIGNO EM UM PERIQUITO-AUSTRALIANO (*Melopsittacus undulatus*)

Resumo

Um periquito-australiano (*Melopsittacus undulatus*) macho com três anos de idade e exoftalmia progressiva esquerda foi submetido à exenteração. Pelo fato de o animal ter vindo a óbito durante o procedimento cirúrgico, o tecido tumoral foi removido por necropsia e a amostra submetida à histopatologia e imuno-histoquímica. Os resultados iniciais foram compatíveis com linfoma. Na imuno-histoquímica os resultados foram negativos para os marcadores CD3 (linfocitos T), CD20 (linfocitos B) e citoqueratina. O marcador para vimentina resultou moderado e variável. Os resultados confirmam a origem mesenquimal maligna do tumor, mas foi inconclusiva para linfoma. Esse é um relato de tumor mesenquimal maligno retrobulbar raro que reforça a necessidade de mais pesquisas sobre doenças neoplásicas nos olhos de aves exóticas.

Palavras-chave: tumor mesenquimal, periquito-australiano, linfoma, oftalmologia veterinária.

Abstract

A three year-old male budgerigar (*Melopsittacus undulatus*) with progressive exophthalmos on the left side, underwent an exenteration procedure. Since the animal died on the surgery, the tumorous tissue was removed during necropsy and its samples were submitted to histopathologic and immunohistochemistry characterization. Results of initial histopathologic examination were compatible with lymphoma. In the immunohistochemistry analysis, results were negative in staining for CD3 (T lymphocytes), CD20 (B lymphocytes) and cytokeratin. Staining for vimentin was moderate and variable. Results confirm a malignant mesenchymal origin of the tumor, but inconclusive for lymphoma. It is a report of a rare retrobulbar malignant mesenchymal tumor which reinforces the need for more investigations about neoplastic diseases in the eye of exotic birds.

Key Words: Mesenchymal tumor, budgerigar, lymphoma, veterinary ophthalmology.

1.1 INTRODUCTION

Several types of neoplasms have been reported in pet birds in the past. The incidence of these neoplasms varies from place among species of pet birds. The eye has been cited as the mean site of neoplastic proliferation in only a few reports. Some of the reported neoplasms in pet bird's eyes include: lymphoma, adenocarcinoma, adenoma, rhabdomyosarcoma, teratoma, osteosarcoma and melanoma (Gandal, 1959; Dukes & Pettit, 1983; Neumann & Kummerfeld, 1983; Tsai et al., 1993; Gulbahar et al., 2005; Simova-curd et al., 2006; Simova-Curd, 2009; Simova-Curd, 2010; Costagliola et al., 2011; Langohr, 2012; Pazdzior, 2012).

Malignant or benign mesenchymal tissue tumors are usually soft in consistency and can affect any organ, being relatively frequent in domestic and wild species of animals (Baba & Câtoi, 2007). Its classification and histological characterization are difficult because the cellular origin of all soft tissue sarcomas is a primitive mesenchymal cell. Those are the most common soft tissue tumors: fibromatoses, fibrosarcomas, equine sarcoids, histiocytomas, neurofibrosarcomas, leiomyosarcomas, rhabdomyosarcomas, liposarcomas, angiosarcomas, synovial cell sarcomas, mesotheliomas and meningiomas (Baba & Câtoi, 2007).

Lymphoma is a lymphoid malignant neoplasm, which originates in solid organs such as liver, spleen and lymph nodes, with a high risk of producing metastasis (Ward, 1996; Sequeira et al., 1999; Nelson & Couto, 2006). In dogs, lymphomas may be mostly linked to genetic factors, whereas in cats the disease may be acquired via infection with the feline leukemia virus in 70% of cases (Ward, 1996; Sequeira et al., 1999; Nelson & Couto, 2006). Lymphomas are divided into five anatomic presentations: multicentric, cutaneous, alimentary, mediastinal and extranodal, incluse in birds (Ward, 1996; Sequeira et al. 1999; Nelson & Couto, 2006). In chickens and some other avian species, the majority of lymphoma cases were related with Marek´s disease virus sorotype 1 infection (Murata, 2012).

Budgerigars (*Melopsittacus undulatus*) are originally from Australia, and currently are most popular pet in the world, after only dogs and cats (Perrins, 2003). The oldest report of neoplasia in budgerigars was in 1959, and after this, few reports have been published, being the pituitary tumors the most common case in this specie (Gandal, 1959; Neumann & Kummerfeld, 1983; Gulbahar et al., 2005; Simova-curd et

al., 2006; Simova-Curd, 2010; Langohr, 2012). There is only one recent report of a retrobulbar tumor in a budgerigar with rhabdomyosarcoma leading to exophthalmos (Gulbahar et al., 2005).

The objective of this report is to describe a spontaneously occurring retrobulbar mesenchymal tumors in a Budgerigar. At the present moment, as far as the authors know, this is the first report of undifferentiated retrobulbar mesenchymal tumors in this species.

1.2 CASE REPORT

A three year-old male budgerigar (*Melopsittacus undulatus*) was referred for ophthalmic evaluation of the left eye to the UFPR´s Comparative Ophthalmology Lab (LABOCO) in Curitiba, Paraná State, Brazil. The owner's perception was that the disease had a two-month history of progressive displacement of the normal eye position. The animal was feeding well and no important behavioral changes were observed. No history of a possible trauma or nutritional disease were reported. Initial diagnoses of the referring veterinarian that evaluated the animal seven days previously, were of a unilateral retrobulbar abscess associated with sinusitis and probably glaucoma.

After clinical history taking part of the owner´s interview the bird was then inspected inside its cage. Both right and left eyes appear to have a good vision, responding to menace gestures from outside the cage. During physical examination, the bird weighted 32g and, no signs of superior or inferior respiratory disease and nutritional deficiency were observed. Ophthalmic examination was performed with a transilluminator and a slit lamp biomicroscope (Hawk Eye; Dioptrix, L'Union, France) that also served as an indirect ophthalmoscope when a 20 diopter lens was adapted in front of the light source. The right eye was normal and had no signs of ocular disease.

The cornea was transparent without evidence of corneal ulceration (negative fluorescein stain). The pupils were able perform both voluntary midriasis and miosis normaly. There were no signs of uveits such as flare, hypopium or hyphema, but bulbar and palpebral conjunctival vessels appeared to be more congested. No epiphora was observed and there was no pain during transpalpebral retropulsion of the eyeball. Retropulsion was not fully achieved on the left eye compared to the right eye. No signs of disease of the posterior segment was observed. No ocular ultrasonography was performed, but the aspect of the eye mass suggested an extraocular neoplasia. After

ophthalmic examination it was concluded that the patient presented a retrobulbar mass on the left side not severely compromising its left eyeball (Figure1).

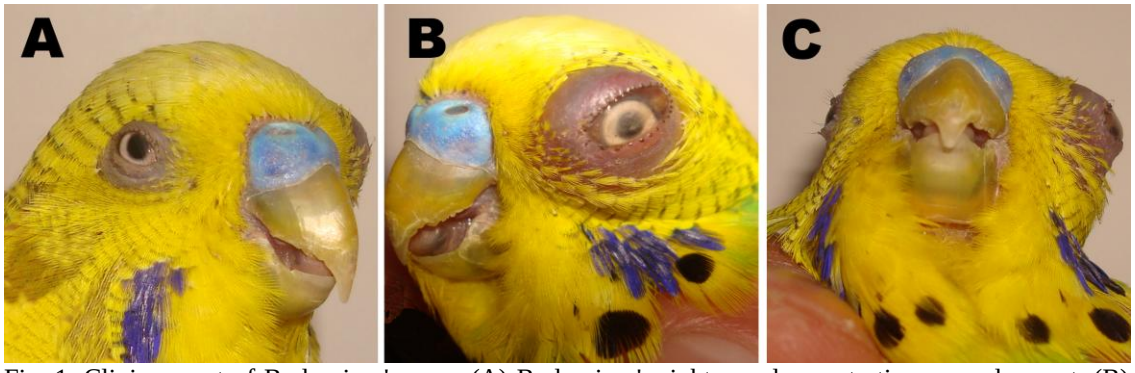

Fig. 1. Clinic aspect of Budgerigar's eyes. (A) Budgerigar's right eye demonstrating normal aspect. (B) Left eye with proptotic aspect with increased of volume and keratitis. (C) Front vision demonstrating volume difference of the eyes.

Initially, the treatment consisted of instillation of topical prednisone acetate 1% eye drops TID (Pred Fort; Allergarn-Frumtost Laboratory, São Paulo, Brazil) and oral administration of a compounded prednisone solution (0.5 mg/kg PO), BID (Drogavet, Curitiba, Brazil) for nine days. During reevaluation nine days later the budgerigar had stop eating and the eyelids were not closing the entire cornea, resulting in superfitial keratitis. The right eye did not show other significant changes. Emaciation was noted on the physical examination. The bird weighted 30g on this visit. A decision to exenterate the affected eye was made. General anesthesia was induced using an intramuscular anesthetic mixture zolazepam and tyletamine 10 mg/kg (Zoletil 50, Virbac Brazil). During exenteration surgery the budgerigar died. Since the procedure was concluded the entire left globe containing the retrobulbar mass was fixed in 10% neutral buffered formalin and submitted for gross and histolopathologic evaluation. The eyeball weighted 0.4 grams and measured 4.5 x 9 x 8 mm, with the cornea measuring 7 x 7 mm and the retrobulbar region was thickened. When the eyeball was cut longitudinally, a whitish compact tissue mass was evident in the retrobulbar region. The mass consistency was soft and was about 6 mm thick in its axial axis (Figure 2A and 2B). Routine histopathologic analysis was performed on the whole globe. Basically, the sample was dehydrated with absolute alcohol, cleared by xylene and included in paraffin block. Five µm-thick sections were cut and then stained with hematoxylin and eosin, Fontana Masson and Toluidine Blue. For immunohistochemistry analysis, sections were made and dewaxed, rehydrated, immersed in 3% hydrogen peroxide for 15 minutes, and then incubated with primary antibodies at room temperature for 1 h.

The following primary antibodies were used: anti-CD3 (rabbit polyclonal, DAKO, Carpinteria, CA, USA); and anti-CD20 (mouse monoclonal, DAKO, Carpinteria, CA, USA). Cytokeratin (clone AE1/AE3 monoclonal Dako, Carpinteria, CA, USA) and vimentin (clone V9 monoclonal Dako, Carpinteria, CA, USA) anti-bodies were used then. The streptavidin-biotin complex kit and diaminobenzidine substrate kits with hematoxylin counterstaining were used to visualize the immunochemical reaction according to the supplier's recommendations.

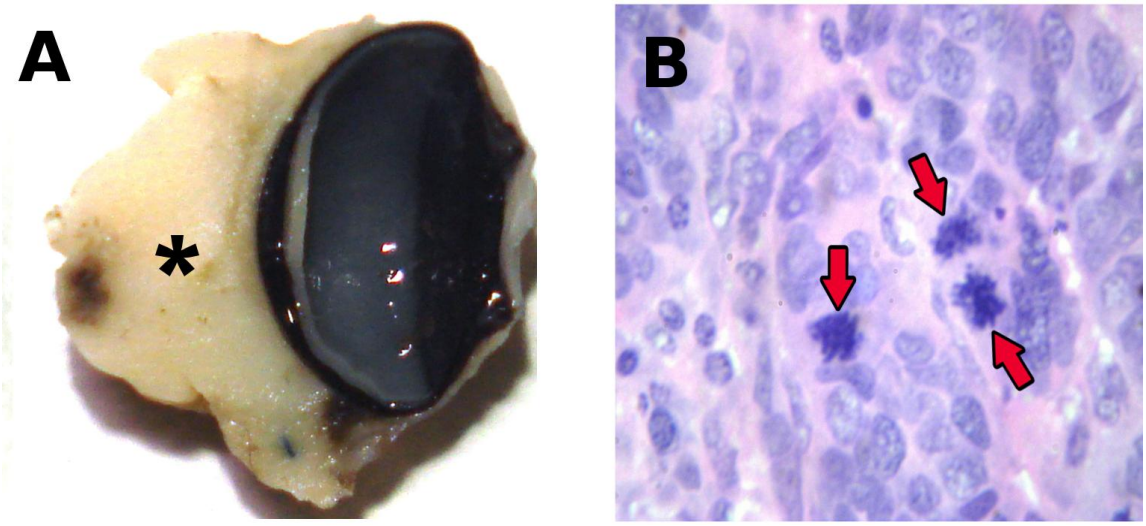

Fig. 2. (A) Macroscopic aspect of the eyeball after enucleation; retrobulbar mass (asterisk). (B) Intense cellular atypia characterized by anisocytosis, anisokaryosis, evident nucleulus and presence of three mitotic figure (arrows) (H.E., x40).

1.3 TEST RESULTS AND DISCUSSION

Histologically there were focally extensive areas of ulceration in the corneal epithelium. There was moderate accumulation of proteinaceous material in the anterior chamber. In addition, a poorly delineated, non-encapsulated and infiltrative mass expanding and replacing the sclera and episcleral tissue adjacent to the optic nerve was observed. The mass was composed of round neoplastic cells arranged in solid sheets and surrounded by scant stroma. The cells presented scant amounts of eosinophilic cytoplasm with distinct cell borders, oval nuclei with finely stipple chromatin, and multiple nucleoli. Cellular pleomorphism was marked and there were approximately five mitotic figures per high power field (Figure 2 B). There were multifocal to coalescing areas of coagulative necrosis throughout the mass associated with

hemorrhage and hemosiderosis. There were few neoplastic cells infiltrating and expanding the iris stroma. No significant changes were seen in the rest of the globe.

Immunohistochemical stains, CD20 (B lymphocytes) and CD3 (T lymphocytes) were both negative. This could signify that this is a double negative lymphoma or that the immunohistochemical reaction did not work on avian tissue. We believe that the latter is more likely since morphologically it resembles very much a lymphoid tumor. On further immunohistochemistry neoplastic cells were negative for cytokeratin (clone AE1/AE3 monoclonal Dako, Carpinteria, CA, USA) and presented variable and moderate cytoplasmic positivity for vimentin (clone V9 monoclonal Dako, Carpinteria, CA, USA) confirming mesenchymal origin of the cells. Fontana Masson and Toluidine Blue resulted negative for melanocitic neoplasm and mastocytoma.

In the veterinary literature, there are no reports about undifferentiated retrobulbar mesenchymal tumors in budgerigars. However, one case report of cutaneous lymphoma in umbrella cockatoo was described, being positive for anti-BLA.36 monoclonal rabbit antibody (90% of neoplastic lymphocytes) and CD3 polyclonal rabbit antibody (Rivera et al. 2009). Other reports described a cutaneous lymphoid tumor in a peafowl and demonstrated that CD3 and CD20 stain were effective in evidence T-cells and B-cells, respectively (Yildirim et al. 2012). Tumor samples from the patient described here in this case report did not stained for CD20 and CD3.
Nevertheles, the morphological aspects of the tumor cells were consistent with a lymphoid tumor.

The slides were reviewed by multiple pathologists and the consensus was that the state of the tissue sample was less than ideal. Probably the eyeball was overfixed in 10% neutral buffered formalin. Overfixation leads to an exaggeration reaction of cross-linking. This damages antigens integrity for immunohistochemistry, mainly by masking protein epitopes and decreasing antigen detection (Joshua et al. 2009). Overfixation can significantly influence immunohistochemistry analysis and may explain why the tissue presented such faint immunohistochemical reaction on the internal control of the samples (i.e. faint cytokeratin reaction on corneal epithelium). Analyzing all results collected in this case, the best diagnostic option from the pathologic point of view was of a undifferentiated malignant mesenchymal tumor. Although the neoplastic cells presented features that were very consistent with a round cell tumor (such as a lymphoid tumor).

Another type of stain that could have been used to completely characterize this tumor would be anti-BLA.36. Iman et al. (1990), described anti-BLA.36 as antibody that recognizes a glycoprotein with an apparent molecular weight of 36 kilodaltons, termed B lymphocyte antigen BLA.36. This marker was found to be specifically expressed on Hodgkin's and human B cell lines including early B progenitor cells, being negative in cell lines representing T cell lymphomas, non-B large cell lymphomas, melanomas and carcinomas. Analyzing this information, two other possibilities are plausible: 1) Mouse CD20 antibody does not work in Psittacine birds B-cells; or 2) Lymphoid tissue found in the present patient was in fact composed of early B-cell, and it could be positive for anti-BLA.36 stain.

Budgerigars and others psittacines are extremely sensitive to blood loss, which may lead to death during surgery (Cubas & Godoy, 2005). Furthermore, in enucleation and exenteration, even a small blood loss could entail death too, as occurred with this patient.

Veterinary literature does not have enough information about prognosis of lymphoma and others neoplasms in pet birds. Latimer et al (1998) described that their patient survived for six months, but in other reports the bird either died during surgery (Gulbahar et al., 2005) or was euthanatized (Coleman & Oliver, 1994), thus, no exact information about prognosis could be taken from this.

This case contributes with more information that certainly will be useful to understand how this neoplastic disease occurs in this exotic species. This case report also reinforces the need for submitting ocular tissue samples to histopathologic analysis after enucleation or exenteration procedures in wild and pet animal ophthalmology.

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CAPÍTULO 2

HEMODYNAMIC OF THE *PECTINIS OCULI* ARTERY IN AMERICAN PEKIN DUCKS (A*nas platyrhynchos domestica*)

Resumo

Objetivo: Mensurar as medidas de velocidade sanguínea da artéria *pectinis oculi* e compará-las com valores encontrados em outras artérias em outras espécies, para propor uma hipótese de sua função.
Métodos: Onze marrecos-de-pequim (*Anas platyrhynchos domestica*) sem afecções oculares foram examinados por ultrassonografia ocular com Doppler. Os índices de resistividade (RI) e pulsatividade (PI) da artéria *pectinis oculi* foram calculados.
Resultados: A média dos valores de RI e PI resultaram respectivamente em: olho esquerdo (0.43 ± 0.06; 0.58 ± 0.10), olho direito (0.37 ± 0.07; 0.47 ± 0.12) e ambos os olhos (0.40 ± 0.07; 0.53 ± 0.12).
Discussão e conclusões: Os baixos valores para RI e PI encontrados na artéria *pectinis oculi* comparada com outros tecidos, sugerem alta atividade metabólica no pécten e isso poderia indicar função nutricional e/ou regulação da pressão intra-ocular.

Palavras-chave: pécten, artéria pectinis oculi, índice de resistividade, índice de pulsatilidade, oftalmologia de aves, medidas de velocidade sanguínea.

Abstract

Objective: To measure blood velocity parameters of the *pectinis oculi* artery and compare with values found in other arteries in other species, to form a hypothesis of its function.
Methods: Eleven american pekin ducks (*Anas platyrhynchos domestica*) without ocular diseases were examined with ocular Doppler ultrasonography. Pectinate artery resistive index (RI) and pulsatility index (PI) were calculated.
Results: The mean of RI and PI values resulted respectively in: left eye (0.43 ± 0.06; 0.58 ± 0.10), right eye (0.37 ± 0.07; 0.47 ± 0.12) and both eyes (0.40 ± 0.07; 0.53 ± 0.12).
Discussion and conclusions: The low RI and PI values found in the *pectinis oculi* artery compared with other tissues suggest a high metabolic activity in pectin oculi and it could indicate a nutritional function and/or intraocular pressure regulation.

Key Words: pecten oculi, pectinis oculi artery, resistive index, pulsatility index, avian ophthalmology, blood velocity parameter.

2.1 INTRODUCTION

Birds (class Aves or clade Avialae) have a unique structure projecting from the top of the optic disc into the vitreous body called the *pecten oculi* (Brach, 1977 ; Kern, 2006; Kiama et al., 2006; Rahman et al., 2010; Micali et al., 2012 ; Mustafa et al., 2013). This structure is pigmented, vascularized and morphologically classified into three types: 1) conical, found in kiwis; 2) vaned, found in ostriches and rheas and, lastly 3) plated, found in all the other avian species including American pekin ducks (Brach, 1977 ; Kern, 2006; Kiama et al., 2006; Rahman et al., 2010; Micali et al., 2012 ; Mustafa et al., 2013). The plated form of pecten has been characterized using histology and electron microscopy and shown to have folds or pleats, held together by a bridge composed of connective tissue containing large vessels surrounded by capillaries and melanocytes (Rahman et al., 2010; Micali et al., 2012 ; Mustafa et al., 2013). Furthermore, there are pecteneal hyalocytes attached to the retinal inner limiting membrane and in direct contact with the convoluted vessels of the pectin (Llombart et al., 2009).

The function of *pecten oculi* was investigated by many authors and several possible functions have been suggested: maintenance of intraocular pressure has been postulated as the main function of the *pecten oculi* in a less recent study (Seaman & Himelfarb, 1963), intraocular pH and pressure regulation (Brach, 1975), stabilization of the vitreous body (Tucker, 1975), reduction of intraocular glare (Seaman & Himelfarb, 1963), blood barrier for the retina and vitreous body (Barlow & Ostwald, 1972) and nutrition for the anangiotic avian retina (Rodriguez-Peralta, 1975). To support this, another study suggests the existence of abundant lymphatic vessels in the avian choroid coat, which could drain intraocular fluids from the posterior segment by a different route from the well-characterized anterior segment aqueous fluid drainage paths in other species (De Stefano & Mugnaini, 1997). Despite all of the previously published work, the most important function and full range of functions of the *pecten oculi* remain uncertain. The complex pecten arteriolar irrigation and its hemodynamic parameters have not been fully characterized yet. The *pectinis oculi* artery was named in a study of the chick embryo, which identified its origin as the circle of Willis and later the internal carotid ophthalmic artery and external ophthalmic artery (also known as the ophthalmic branch of the stapedius artery) (Baumel, 1993; Hiruma, 1996), which is the most important and thickest artery supplying the eye in the chick (Hiruma, 1996). Moreover,

Baumel in Nomina Anatomica Avium (NAA), also names the external ophthalmic artery as the origin of the *pectinis oculi* artery (Baumel, 1993).

Ultrasonography is an exceptional and noninvasive option to evaluate the *pecten oculi´*s blood vessels. However, to the best of the authors' knowledge, no studies of the pecten's ocular structures have been performed using this technique. Color and pulsed Doppler imaging ultrasonography allows for the evaluation of blood velocity parameters such as peak systolic velocity (PSV), end diastolic velocity (EDV), pulsatility index (PI) and resistive index (RI) (Greenfield et al., 1995; Gellat-Nicholson et al., 1999).

Several studies have been conducted to map orbital and ocular arteries and to measuring their blood velocity parameters in various species, but no investigation have been performed in the *pecten oculi* of birds (Gellat-Nicholson et al., 1999; Brooks, 2007; Carvalho, 2009; Yang, 2011). Thus, the objective of this investigation was to evaluate the blood flow in the *pecten oculi* artery, to contribute to the knowledge of the *pecten oculi*'s function.

2.2 MATERIAL AND METHODS

2.2.1 *Animals*

Eleven three-year old American pekin ducks (*Anas platyrhynchos domestica*), two males and nine females, with a mean weight of 3.0 ± 0.3 kg, were evaluated. Before the ultrasonographic procedures, the animals were evaluated using a slit lamp biomicroscope (Hawk Eye; Dioptrix, L'Union, France) and indirect ophthalmoscope (Eye Tech; São Paulo, Brazil) to ensure the ducks did not have any ocular abnormalities. This study was approved by the Federal University of Paraná´s Animal Welfare Committee and was conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2.2 *Ocular Doppler Ultrasonography*

For this procedure, all animals were physically restrained with a blanket. Blood velocity parameters in the *pecten oculi* were evaluated using an ultrasound system (MyLab 30 – Esaote, Genova, Italy) coupled with a high-frequency 12 MHz ultrasound linear transducer. After instillation of one drop of a topical anesthetic eyedrop formulation (proximetacaine 0.5% - Anestalcon - Alcon do Brasil, São Paulo, Brazil),

ultrasonic gel (Carbogel, São Paulo, Brazil) was applied over the corneal surface. Then, the ultrasound transducer was perpendicularly positioned in a horizontal plane, from 3 to 9 o'clock. The long axis of the transducer was placed on top of the corneal surface (transcorneal technique), with the marker pointing nasally and inclined 20º. First the pecten's base was observed by B-mode ultrasound, near the optic nerve. Then the *pectinis oculi* artery was located using the power Doppler mode to inspect the blood flow.

Resistive index was determined by RI=(PSV-EDV)/PSV, and pulsatility index was determined by PI=(PSV-EDV)/TAMX, where TAMX is the time average of max velocity (Greenfield et al., 1995; Hiruma, 1996; Gellat-Nicholson et al., 1999). Values of RI can vary from 0 to 1, with high values indicating less blood delivery and thus slow tissue metabolism, and low values indicating greater blood flow and faster tissue metabolism (Gellat-Nicholson et al., 1999).

2.2.3 *Statistical Analyses*

Descriptive statistics of blood flow parameters is presented. *T*-tests were performed to compare values obtained on right and left eyes. Differences were considered significant when $P<0.05$.

2.3 RESULTS

The flow towards the transducer was demonstrated in red color and the blood flow away from the transducer in blue (Figure 1A). As the power Doppler system was detecting blood flow, spectral Doppler sampling volume was placed in the center of the artery´s image (Figure 1B). Three waves were chosen, velocities (PSV and EDV) and indices (RI and PI) were measured from each artery flow (Figure 1C), and the results were averaged.

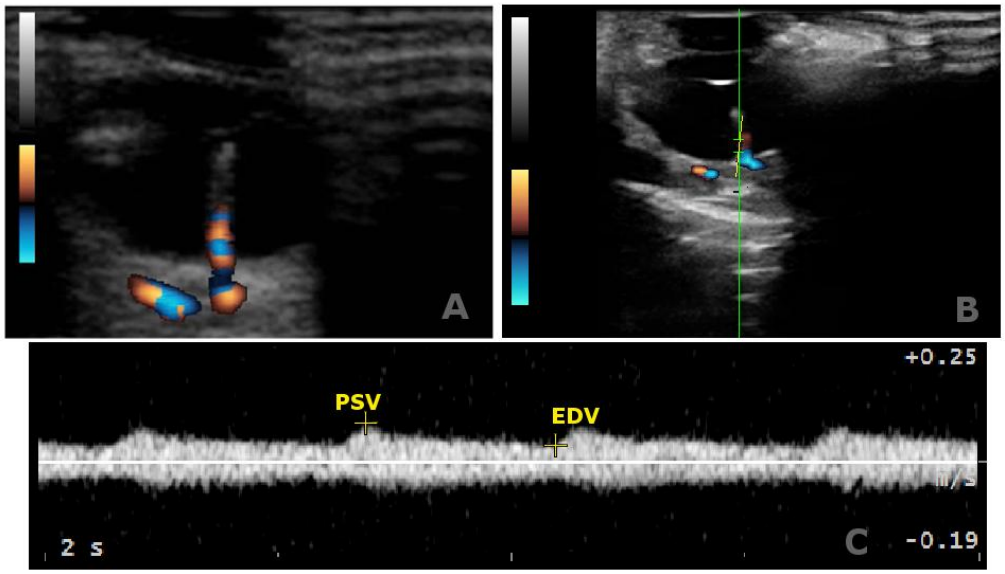

Figure 1. Power and spectral Doppler images. (A) *Pecten oculi* blood flow detected by power Doppler; (B) Spectral Doppler pointing *pecten oculi* basis; (C) Blood flow waves with PSV and EDV indicated. PSV – Peak Systolic Velocity; EDV – End Diastolic Velocity.

Peak systolic velocity, EDV, RI and PI values of *pectinis oculi* artery are shown in Table 1. No significant differences between right and left eyes were found (P>0.05).

Table 1. *Pectinis oculi* artery blood velocity parameters of eleven American pekin ducks.

Measurements	Minimum-Maximum	Mean	SD	SE
Left eye OS (n=11)				
PSV (cm/s)	5.17-12.07	8.16	2.09	0.63
EDV (cm/s)	2.47-6.9	4.66	1.39	0.42
RI	0.33-0.53	0.43	0.06	0.02
PI	0.40-0.73	0.58	0.10	0.03
Right eye OD (n=11)				
PSV (cm/s)	4.87-10.80	8.08	1.76	0.53
EDV (cm/s)	2.73-7.17	5.08	1.28	0.39
RI	0.27-0.52	0.37	0.07	0.02
PI	0.32-0.74	0.47	0.12	0.04
Both eyes OU (n=22)				
PSV (cm/s)	4.87-12.07	8.12	1.89	0.57
EDV (cm/s)	2.47-7.17	4.82	1.32	0.40
RI	0.27-0.53	0.40	0.07	0.02
PI	0.32-0.74	0.53	0.12	0.04

SD: standard deviation; SE: standard error; PSV: peak systolic velocity; EDV: end diastolic velocity; RI: resistive index; PI: pulsatility index.

2.4 DISCUSSION

Ultrasonography is a non-invasive examination method used to investigate the majority of organs, including shape, inner structure, and blood parameters by Doppler (Carvalho, 2009). Doppler ultrasonography is based on the reflection of the sound waves off flowing red and white blood cells. As a convention flow towards the transducer is depicted in red and flow away from the transducer in blue (Gellat-Nicholson et al., 1999; Brooks, 2007; Carvalho, 2009; Yang, 2011). Furthermore, it is possible to measure blood velocity parameters by pulsed Doppler, which provides information about flow features as RI (Gellat-Nicholson et al., 1999; Brooks, 2007; Carvalho, 2009; Yang, 2011).

A number of orbital and ocular blood flow parameter investigations have been published in the veterinary and medical ophthalmology literature (Tokoro, 1972; Greenfield et al., 1995; Gellat-Nicholson et al., 1999; Brooks et al., 2007; Carvalho, 2009; Yang et al., 2011). However, there are no published studies about the flow of orbital, ocular or pecten blood vessels in any avian species (Tokoro, 1972; Greenfield et al., 1995; Gellat-Nicholson et al., 1999; Brooks et al., 2007; Carvalho, 2009; Yang et al., 2011). Assumptions about a tissue's metabolism can be made from its blood supply's PDV, EDV, RI and PI parameters (Nelson & Pretorius, 1988; Martinoli, 1998; Carvalho, 2009). Both RI and PI have been used to evaluate artery flow in many organs and tissues in a variety of studies in cat (Carvalho & Chammas, 2011; Reis et al., 2014), chicken (Barua et al., 2007), dog (Gellat-Nicholson, 1999; Lamb et al. 1999; Carrillo et al.,2011; Lee et al. 2014) and rabbit (Tokoro, 1972; Abdallah et al., 2010; Yang et al., 2011). Tissues with higher metabolisms have lower RI and PI values, whereas tissues with low or slower metabolism have higher RI and PI values (Nelson & Pretorius, 1988; Martinoli, 1998; Carvalho, 2009). For instance, in cats the femoral artery, which supplies tissues with lower metabolisms, has high RI and PI values 0.78 ± 0.28, respectively (Reis et al. 2014), whereas the renal artery, which supplies the highly metabolically active kidney, has low RI and PI values (0.54 ± 0.07, respectively) (Carvalho & Chammas, 2011).

Concerning ocular blood flow, the long posterior ciliary artery (LPCA) is the main artery that supplies the choriocapillaris layer (Abdallah et al., 2010). The lowest RI reported for animals, in our research, was that of the LPCA in the rabbit (0.09 ± 0.05)

(Abdallah et al., 2010). The low RI value found in the LPCA of rabbits demonstrates the metabolic importance of the choriocapillaris layer to supply the noncentral region of the paurangiotic retina, unlike the greater RI values found in the holangiotic retina of dogs (Bill, 1985; Gellat-Nicholson, 1999; Samuelson, 2006; Abdallah et al., 2010). The RI value for the canine LPCA, as reported by Gelatt-Nicholson et al was 0.51 ± 0.006, which was higher than that reported for the LPCA of rabbit (Gellat-Nicholson, 1999). This suggests that the choriocapillaris in dogs does not require as high a blood flow (as that of rabbits) because the retina's nutrition comes from the retinal blood vessels.

Mean RI and PI values found in the *pectinis oculi* artery were higher than those reported for the long posterior ciliary artery in rabbits and similar to those reported in the ciliary body vasculature of dogs. This could suggest that the *pecten oculi* artery is not as important to the retinal metabolism of the avascular avian retina as the long posterior ciliary artery is for the choroidal vasculature of the rabbit. Nevertheless, the nutritional/metabolic importance of the *pecten oculi* cannot be completely rejected because its RI and PI are still considered to be within the "low" range (Carvalho, 2009) and thus supply a tissue with a high metabolic rate. If that is the case, retinal arteriolar irrigation via pectin in birds would constitute a unique method of nutrition delivery to a tissue because substances would have to diffuse through the vitreous to reach the retina.

If the pecten is responsible for the aqueous humor production and intraocular pressure (IOP) maintenance in the posterior segment, there should be an additional drainage system near this site. There are many histologically visible lacunas in the avian choroid, which were suggested to be lymphatic vessels due to their thin endothelium, absence of well-delimited basal lamina muscular tunica, innervation and acellular material filling their lumens (De Stefano & Mugnaini, 1997). Furthermore, these lacunae become smaller and less numerous near the optic nerve, *pecten oculi* and iridotrabecular angle and therefore are not a part of Schlemm's canal (De Stefano & Mugnaini, 1997). This morphological observation and our results of similar RI and PI of the *pectinis oculi* artery to the ciliary body arteries in dogs could signify that the *pecten oculi* has secretory functions as well.

In this study we have demonstrated selected blood flow parameters of the *pectinis oculi* artery. Similar values were found in arteries that supply the ciliary body (long posterior ciliary, short posterior ciliary and anterior ciliary arteries) in dogs and rabbits (Gellat-Nicholson, 1999; Abdallah, 2010). The values obtained for the *pecten oculi* do not directly determine the exact function of this structure. Nevertheless, similar

parameters could signify similar functions, in this case, aqueous humor formation and consequently maintenance of IOP (Seaman & Himelfarb, 1963; Brach, 1975; Rodriguez-Peralta, 1975). The existence of lymphatic vessels in the choroidal coat to drain intraocular fluid from the posterior globe could suggest that there exists some structure in the posterior segment to produce these fluids.

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3.1 Aprovação no Comitê de Ética do Setor de Ciências Agrárias da UFPR

Universidade Federal do Paraná
Setor de Ciências Agrárias
Comissão de Ética no Uso de Animais – CEUA SCA

CERTIFICADO

Certificamos que o protocolo no. 031/2014, referente ao projeto "Hemodinâmica da artéria pectiniana em marrecos-de-pequim (*Anas Platyrhynchos*)", sob a responsabilidade de Thiago Alegre Coelho Ferreira, na forma em que foi apresentado (uso de 12 marrecos-de-pequim), foi aprovado pela Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias, em reunião realizada dia 31 de julho de 2014.

CERTIFICATE

We certify that the protocol number 031/2014, regarding the project "Pectinate artery hemodinamic in white mallard (*Anas platyrhynchos*)", under Thiago Alegre Coelho Ferreira's supervision, in the terms it was presented (use of 12 mallard), was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of Paraná, Brazil) during session on July 31st, 2014.

Curitiba, 31 de Julho de 2014.

Ricardo Guilherme D'Otaviano de Castro Vilani
Presidente

Ananda Portella Félix
Vice-Presidente

Comissão de Ética no Uso de Animais
Setor de Ciências Agrárias
Universidade Federal do Paraná.

3.2 VITA

Formado em medicina veterinária pela Universidade Estadual de Santa Catarina em 2008, se aperfeiçoou em oftalmologia veterinária pela Universidade Federal do Paraná em 2009 e em ciências básicas aplicadas à oftalmologia veterinária no Centro de Oftalmologia para Animales em Buenos Aires, Argentina. Especializou-se em anestesiologia veterinária pela Universidade de Jaguariúna e Instituto Bioethicus em 2010, ano em que foi habilitado pela Sociedade Latino-Americana de Emergência e Cuidados Intensivos em medicina veterinária intensiva.

Ingressou no Programa de Pós-Graduação em Ciências Veterinárias da UFPR em 2013, participando de projetos de doutorado (concluído) de Ana Carolina da Veiga Rodarte de Almeida e de mestrado de Renan Shiebel Medeiros (concluído). Colaborou para o trabalho de iniciação científica de Fernanda Cardoso Cancelli Vieira. Atuou como organizador, ouvinte e palestrante do Grupo de Estudos em Oftalmologia Comparada da UFPR.

Foram submetidas duas publicações, sendo uma na revista Pesquisa Veterinária Brasileira (Qualis A2) e outra na revista *Veterinary Ophthalmology* (Qualis B2). Outros estudos de revisão e metanálise resultarão em posteriores publicações para o ano de 2015.